Chapter III. WATER COLLECTION PROTOCOLS

Section A. Water Quality Sondes: Calibration, Maintenance, & Use

Part 1. Sonde Calibration and Maintenance

The following procedures are a basic overview of YSI calibration for an YSI 600XL Sonde/650 MDS display combination and Hydrolab Quanta G. These instructions are meant as a quick reference guide to the steps involved in calibrating a sonde and do not supplant the manufacturers' operation manual. Consult the owner's manuals for specifics or information on configurations other than these and for details on maintenance and trouble-shooting. These procedures assume the user has a basic knowledge of the instrument.

These directions are not intended for first-time users. Individuals with no prior experience should calibrate with the assistance of an experienced user.

All calibration adjustments are documented on a permanent Sonde Calibration Sheet. The date and time of calibration, name of the calibrator, the identification number of the unit, battery voltage and all adjustments/maintenance must be documented (***see example in Figure 35 on the next page***).

Note: Rinsing the probe is a procedure that is frequently performed during calibration. To rinse the probe, install the calibration cup (which is the same as the storage cup on YSI and Quanta G sondes) and add about 1/2 cup of rinse solution, as specified in the directions (usually deionized (DI) or distilled water). Seal the open end of the calibration cup with the screw cap or rubber lid and shake the probe for 30 seconds. Discard rinse water and repeat according to directions.

All calibrations are performed with the probes in the pointing upward and at temperatures as close to room temperature as possible (25^{0}C). If calibration does not occur at room temperature (*e.g.,* a field calibration) then every attempt should be made to temperature adjust the calibration solutions according to the manufacturers' guidelines presented in Figure 36, Figure 37, & Figure 38 on pages 115, 116, & 117 respectively.

Sonde ID: _____ Date: _____ Time: _____ Calibrator's Initials: _____

Battery Check (V): _____ Field User's Initials (if different from Calibrator): _____

Temperature (°C): _____ *Temperature must be recorded for all Calibrations (even single probe calibrations)!*

DO Probe Calibration-*Check the circles after considering each of the following.*

- ○ *Be sure to check the age of the DO probe. It should be no more than 2 years old for 600 XL YSIs (5-7 years for 556 YSIs).*
- ○ Is the D.O. Membrane > 30 days old? If so, it needs to be changed regardless of the amount of use it has seen.
- ○ *For those sondes that have it, is the D.O. charge <100? If not, then resurface the probe's electrodes with sandpaper.*

Atmospheric Pressure (mm Hg): _____ □ New DO Membrane Installed

Initial % Sat: _____ Final % Sat: _____

Initial DO (mg/L): _____ Final DO (mg/L): _____ DO Charge: _____

Specific Conductance Probe Calibration-*Check the circles after considering each of the following.*

- ○ *For best accuracy, Specific Conductivity should be calibrated according to the expectations of the streams/sites you will be sampling. It may be necessary to recalibrate between streams/sites due to extreme differences between streams/sites (e.g., 1000 vs. 10000 µmhos/cm).*
- ○ *Be sure to check the age of the Specific Conductivity probe. It should be no more than 3 years old.*

Conductivity Solution (µmhos/cm): _____

Initial Sp Cond (µmhos/cm): _____ Final Sp Cond (µmhos/cm): _____

Low-End Sp Cond Check (i.e., <5 µmhos/cm)
Solution: □ Deionized or □ Distilled Water Sp Cond (µmhos/cm): _____

Monthly Mid-Range Sp Cond Check (e.g., 500 or 1000 µmhos/cm)
Conductivity Solution (µmhos/cm): _____ Sp Cond (µmhos/cm): _____

pH Probe Calibration-*Check the circles after considering each of the following.*

- ○ *If the streams/sites you will be sampling are expected to fall within the same pH range (i.e., 4-7 vs. 7-10), pH should be calibrated using a two-point calibration for best possible accuracy. Should a stream site fall outside of the calibrated pH range, then recalibration is necessary. If expectations are unknown, use a three-point calibration.*
- ○ *Be sure to check the age of the pH probe. It should be no more than 12 to 18 months old.*

Initial pH (7): _____ Final pH (7): _____ mV (7): _____ *(0 +/- 30 mV)*

- ○ *The pH 7 mV should be in a range of +/- 30 mV. Between +/- 30-50 mV, the probe is still useable, but should be monitored closely for irregularities with the ranges of mV on the 10 and/or 4 buffer solutions. After +/- 50mV, the probe should no longer be used.*

Initial pH (10): _____ Final pH (10): _____ mV (10): _____ *(-180 +/- 30 mV)*

Initial pH (4): _____ Final pH (4): _____ mV (4): _____ *(+180 +/- 30 mV)*

Is the Nernst Equation OK? □ Yes or □ No

Probe Maintenance Info

Probe Installed: □ New or □ Used Probe Type: □ pH □ DO □ Temp/Sp. Cond.

Probe Model #: _____ Probe S/N: _____

Calibration/Maintenance Notes: _____

_____

_____

Figure 35. Example of Sonde Calibration Log Sheet

YSI 600XL Sonde/650 MDS Display Unit Calibration

These directions are very similar to the older Scout 2 Hydrolab and newer Quanta G directions. However, individuals with no prior experience should calibrate with the assistance of an experienced user.

1) YSI Display Unit

The YSI display unit uses a series of escapable menus in conjunction with several keys in the calibration process. Become familiar with the **Enter** key (which looks like a left arrow), escape, scroll, and alpha-numeric keys as these will be the most often used.

Maintenance of YSI Display Unit

The YSI display unit runs on a 4 alkaline C-cell battery system contained within the display unit. The battery power left is displayed on the screen.

Also of importance is the fact that the results of calibration for YSI units are stored in the sonde itself, not in the display unit. Switching the sonde and display units will not affect calibration. This may be especially helpful as one can calibrate several sondes with only one display unit as others may be recharging.

The display unit also features a Date/Time and an auto-shutoff function, which may be modified by selecting "System Setup" in the Main Menu and then selecting the appropriate function to modify.

2) Dissolved Oxygen

A) DO Probe Calibration

Note: With some of the newer sondes (2004-Present), you need to run the sonde just as if it was in the stream to get the initial or pre-calibration DO readings and then go thru the following steps to calibrate DO and get the post-calibration readings.

1. Remove the threaded lid to the calibration cup. Unlike the Hydrolab sondes, it is not necessary to dry the membrane on the D.O. probe by blotting it with a soft cloth or tissue, but rather only make sure that the membrane is **not inundated with water. In fact, YSI recommends against touching DO membranes when replacing or servicing them. There is a potential for oils or dirt to affect O_2 diffusion through the membrane.** Also, check the membrane for wrinkles, tears, bubbles, dirt, etc. and replace membrane, if necessary.

2. Reattach the calibration cup to the sonde and add no more than 1/8-inch of DI (deionized) or distilled water. Try not pour water on the membrane, but if it does get wet, just make sure that the membrane is not totally inundated with water. Make certain that the DO and Temperature probes are not immersed in water.

3. Cover the calibration cup with the lid and engage only 1 or 2 threads. An alternative to cover the calibration cup with a moist paper towel, then place the lid on upside down on top of the cup with a small weight on top of the lid.

4. Turn on the unit and let sit for about 10 minutes so that the air inside the cup will saturate with water and come to thermal equilibrium.

   Note: If the sonde is from a newer manufacture year (2004-Present), you may need to do one-time adjustment of the sonde settings so that it will give you the initial or pre-calibration readings before continuing the calibration procedure. The manufacture year can be determined by reading the first two digits of the sonde's serial number (*e.g.,* 04=2004). If this is the case, this can be fixed by deactivating the Autosleep RS232 function in the following section of the menu: Sonde Menu > Advanced > Setup > Auto sleep RS232. Toggle the function off by pressing the enter button**.**

5. Turn on the unit and use the **Up** or **Down** keys to scroll to "Sonde Menu" and press **Enter**. Select "Calibrate" and press **Enter**.

6. Scroll to select "Dissolved Oxy" and press **Enter**.

7. Select "DO %", press **Enter**. One must keep in mind that this is actually calibrating based on O_2 air saturation, not water saturation.

8. Type in the Barometric Pressure displayed by the unit in the bottom right of the screen using the alpha-numeric pad; press **Enter**. Wait for both temperature and DO readings to stabilize; this may take up to 40 seconds (after waiting the initial 10 minutes for water vapor equilibration in the cup). The upper right of the screen should have the word "Calibrate". Record the initial or pre-calibration DO, temperature, and % air saturation on the Sonde Calibration Log Sheet. If the upper right of the screen has the word "Continue" instead of "Calibrate" then calibration has already occurred and the readings given are the final or post-calibration readings. This can be avoided for future calibrations by deactivating the Autosleep RS232 function in the following section of the menu: Sonde Menu > Advanced > Setup > Auto sleep RS232. Toggle the function off by pressing the enter button. This will permanently allow the initial or pre-calibration readings will be available prior to calibration.

9. Press **Enter** to finish calibration. Record the final or calibrated DO and % air saturation in log book.

10. The final % air saturation should be within the range of 98% air saturation at the lowest WV elevations to 83% at the highest WV elevations (+/– 2 %). The probe should typically not read above 100% air saturation as this only occurs at sea level. A 100% reading may also be caused at low WV elevations by a high-pressure front or unusual weather in the area. Consult the attached sheet for air saturation values that should be found a different elevations or Appendix D (page 227) from the YSI operating manual (***see Table 8 below***). YSI probes may be calibrated at lower elevations and then brought to a higher elevations and still be accurate. However, calibration at an extreme elevation and transport to a lower elevation may require a recalibration at the lower elevation. If the barometer reading is extremely unusual for your local elevation, the internal barometer may require recalibration in the lab by a person familiar with that procedure.

11. The upper right of the screen will say "Continue". Press **Enter**. And it will take you back to the DO Calibration Menu.

Table 8. From Appendix D Table 2 of the YSI operating manual (page 227)

Pressure (mm Hg)	Altitude (ft.)	Expected % Saturation (+/- 2 %)
760	0	100
752	278	99
745	558	98
737	841	97
730	1126	96
722	1413	95
714	1703	94
707	1995	93
699	2290	92
692	2587	91
684	2887	90
676	3190	89
669	3496	88
661	3804	87
654	4115	86
646	4430	85
638	4747	84
631	5067	83

Elevation at Harpers Ferry=249 ft. and at Spruce Knob=4862 ft.

B) DO Probe Maintenance

The membrane on the DO probe should be examined for fouling and bubbles before calibration and during use. If the membrane is torn, dirty or wrinkled, or if there are bubbles under the membrane, the membrane must be replaced. YSI recommends the membrane be replaced at least every 30 days. **The membrane should be replaced 24 hours before use or calibration to allow time for the new membrane to relax. If, in an emergency, a DO probe must be used before the**

complete 24 hour relaxation period has lapsed, a minimum of 30 minutes must elapse prior to use. In addition, significant drift in its response should be expected due to shifting tension in the membrane. Therefore, the probe should be calibrated every hour it is used until the full 24 hour relaxation period has passed. For most TMDL and other short-duration sampling events, this means the user will most likely need to recalibrate the DO probe before every site.

To replace the membrane, remove the O-ring and old membrane and shake the remaining electrolyte (KCl solution) out of the probe. New KCl is available as an undissolved solid pre-aliquoted in a bottle and provided with each new DO probe or in an YSI maintenance kit. This bottle should be filled with DI or distilled water to the **neck** to provide the proper working concentration. Add a few drops of fresh KCl solution to the probe. The tip of the probe should be filled to create a positive meniscus (looks like an "outie"), and should be free of bubbles. Hold new membrane between thumb and probe body. Use your free hand to stretch the membrane up, over, and down the opposite side of the probe. Secure the loose end with your forefinger. Roll the O-ring over the tip of the probe without touching the membrane with your finger. Cut off excess membrane. Document any membrane replacement on the Sonde Calibration Log Sheet.

Caution: The KCl solution used under the DO membrane is especially corrosive to the electrical contacts on the probes and should not be allowed to contact these electrodes or come in contact anywhere near open probe ports when a probe is being removed or installed.

C) DO Probe Diagnostic

To check the quality of the calibration or diagnose a potential problem with the DO probe, an advanced function called DO charge may be used.

1. Press **Esc** to get the Main Menu.

2. Use the **Up** or **Down** keys to scroll and select “Report”.

3. Scroll down and select “Dochrg” and press **Enter**. When this is done, the symbol to the left of “Dochrg” should change from an empty to a black circle.

4. Press **Esc** twice to get the 650 Main Menu. Scroll up to “Sonde Run” and press Enter.

5. A new parameter should be visible on the screen called “DOc”. If the probe is in adequate condition and calibrated successfully, the number should range from 25 - 75 with a score of 50 being optimum.

6. If the probe reads in this range, then simply repeat this procedure to turn off the DO charge function (the black circle will change back into an empty circle).

If the DO charge is in the low end of the range or below this range, the KCl solution under the membrane may be contaminated with water. In this case the membrane and solution should be replaced.

If the DO charge is in the high end of the range several things may be wrong. First, the highly malleable Au electrode may be distorted or the silver-plating on the electrode may be "tarnished" and gray looking. In this case, the electrode may be reconditioned by buffing it using one of the **YSI provided buffing discs only**. THIS SHOULD ALSO BE DONE ONLY WITH STRICT ADHERENCE TO THE DIRECTIONS PROVIDED IN THE MANUAL FOR USING THIS BUFFING DISC ON THE PROBE SURFACE. IT MAY BE NECESSARY TO CONSULT WITH AN YSI REPRESENTATIVE BEFORE ATTEMPTING THIS ACTION. **YSI recommends running newly-buffed probes for 10-15 minutes continuously to realize good stability.**

A second possible cause of a high DO charge reading are cracks around the electrodes as a result of drying and rewetting of the surface. If this is the case, then the DO probe may need to be replaced.

YSI's 6562 Clark cell DO probes have an expected lifetime of 3-5 years from the date of manufacture. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe has passed its expected lifetime, it may simply be too old to give proper calibration and readings.

D) DO Probe Accuracy

The DO probe accuracy is +/- 0.2 mg/L (or ppm) O_2 or 2% of the reading (whichever is greater). The range for % saturation is or +/- 2 % or the reading or Air Saturation (whichever is greater).

3) Conductivity

A) Conductivity Probe Calibration

1. Remove the lid on the calibration cup and use the special brush designed to fit inside the conductivity probe's 2 end ports, vigorously scrub each port 5-10 times.

2. Rinse the probe 3 times with DI or distilled water.

3. Rinse the probe 2 times with a small amount of **fresh** conductivity standard in the **1000-5000** microSiemens or μS (also known as micromhos or μmhos/cm) range. The exact concentration of the standard will be written on the bottle. The concentration of solution used should be dictated by the conductivities expected to be encountered during the use of the instrument during the week.

4. Fill cup with conductivity standard to within a centimeter of the top of the cup. Make sure that there are no bubbles in the measurement cell of the specific conductance sensor (*e.g.* gently inverting the sonde several times). Record the concentration of standard used to calibrate on the Sonde Calibration Log Sheet.

5. Press **Escape**. Scroll to **conductivity**; press **Enter**.

6. Scroll to **SpCond**; press **Enter.**

7. Type in concentration of standard in milliSiemens (not microSiemens). For example, 1000 microSiemens = 1.000 milliSiemens. Press **Enter**.

8. Allow the reading to stabilize, (a maximum of one minute, though the conductance probe response time is usually the fastest of all probes). Record the initial or pre-calibration readout on the Sonde Calibration Log Sheet. Press **Enter** to calibrate and record the final or calibrated readout on the Sonde Calibration Log Sheet. Press **Enter** again to continue back to the conductivity menu.

9. Now, after at least 3 rinses with DI or distilled water, exit from the calibration menu

10. Enter the discrete sampling mode (Sonde Run from the Main Menu), and conduct a **Low-End Specific Conductance Check** using either DI or distilled water (expected conductance =< 5 microSiemens). This Low-End Specific Conductance Check should be conducted at least once a week during

calibration. Indicate the solution used and the Low-End conductivity solution reading on the Sonde Calibration Log Sheet.

11. At least once a month, conduct a **Mid-Range Specific Conductance Check** using either a known 500 or 1000 microSiemens solution, depending on the solution used to calibrate the conductivity probe. Indicate the conductivity of the solution used and the Mid-Range conductivity solution reading on the Sonde Calibration Log Sheet.

B) Conductivity Probe Maintenance

The openings that allow fluids to access the conductivity electrodes should be cleaned regularly (once a month at most) using the small acrylic brush included in the YSI calibration kit. Dip the brush in clean water and insert it into each hole 20-30 times. A mild detergent may be used with the brush, if deposits have formed on the electrodes.

C) Conductivity Probe Diagnostic

The conductivity probe on an YSI sonde can be checked using a function called Cal Constants.

1. Press **Esc** to get the Main Menu.

2. Scroll down and select “Advanced” and press **Enter**.

3. Scroll down and select “Cal Constants” and press **Enter**. The reading next to the “Cond:” should range from 4.5 – 5.5. IF THE READING IS NOT WITHIN THIS RANGE CONSULT THE YSI OPERATION MANUAL OR AN YSI REPRESENTATIVE.

4. To escape from this screen, press Esc repeatedly until the Main Menu appears.

YSI’s Temperature/Conductivity probes have an expected lifetime of 2-3 years from the date of manufacture. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe has passed its expected lifetime, it may simply be too old to give proper calibration and readings. **Note that YSI 556 sondes have Conductivity Probes that are not interchangeable with the YSI 600 series.**

D) Conductivity Probe Accuracy

The Conductivity probe accuracy is +/- 0.5% +/- 1 μS/cm. For example, a solution that is 1000 microSiemens, the range would be 1000 x 0.005 +/- 1 microSiemen or 5 +/- 1 microSiemen.

4) pH

The pH probe on an YSI sonde can be calibrated using one three methods:

1) One-Point
2) Two-Point
3) Three-Point

If you know what side of neutral (*i.e.,* pH=7) the majority of the streams you are going to be sampling will be on, it is recommended that you use a Two-Point calibration. A Three-Point Calibration may seem to be advantageous since it covers both sides of neutral (*i.e.,* acidic and alkaline), but it has been observed that accuracy can suffer, especially near or beyond the two endpoints (*i.e.,* 4 and 10 pH). Since the Three-Point calibration covers all three pH buffers, we will describe it below as an example.

A) pH Probe Calibration (Three-Point calibration)

1. Press **Escape** to get to Calibration mode.

2. Rinse probe three times with DI or distilled water.

3. Scroll down to **ISE1 pH**; press **Enter.**

4. Scroll down to **3 Point**; press **Enter.**

5. Rinse probe twice with DI or distilled water and once with 7.0 buffer solution.

6. Fill calibration cup with 7.0 buffer solution to within a centimeter of the top of the cup.

7. Because the exact pH of a buffer varies (depending on its constituents) with its temperature, a table provided by the manufacturer must be used to determine the exact current pH of the buffer solution. ***Refer to Figure 36 below or the chart hanging in the lab for the exact pH of a given buffer solution at the current temperature of the room.***

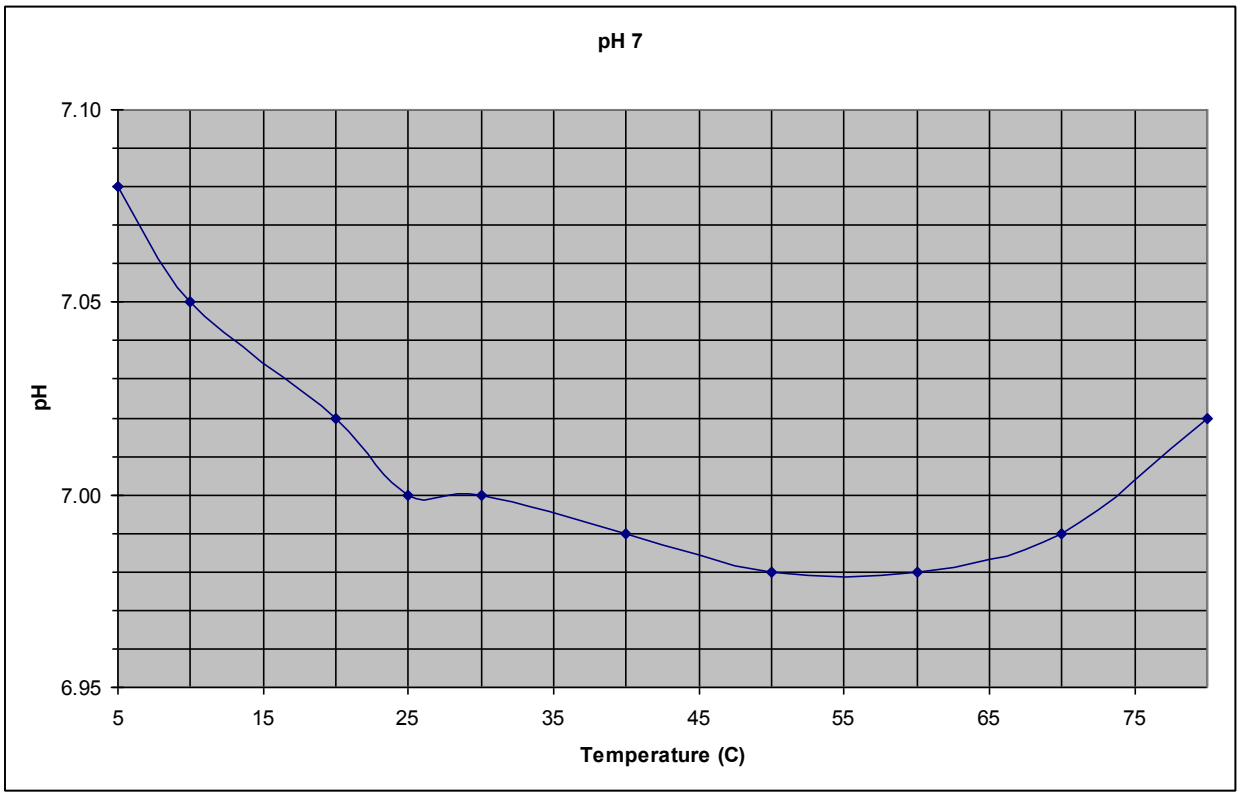

Figure 36. Temperature/pH curve for pH 7 Buffer Solution

8. Type in the exact pH for the 7.00 buffer solution for the current temperature; press **Enter**. Allow readout to stabilize (approximately one minute).

9. Record the initial or pre-calibration readout. Press **Enter** to calibrate.

10. Record the final or calibration readout and press **Enter** again.

11. Rinse probe 2 times with DI or distilled water and once with pH 10.00 buffer solution.

12. Fill calibration cup with 10.00 buffer solution to within a centimeter of the top of the cup.

13. Determine the exact current pH of the 10.00 buffer solution from the table provided by the manufacturer or in ***Figure 37 below*** as in Step 7 above.

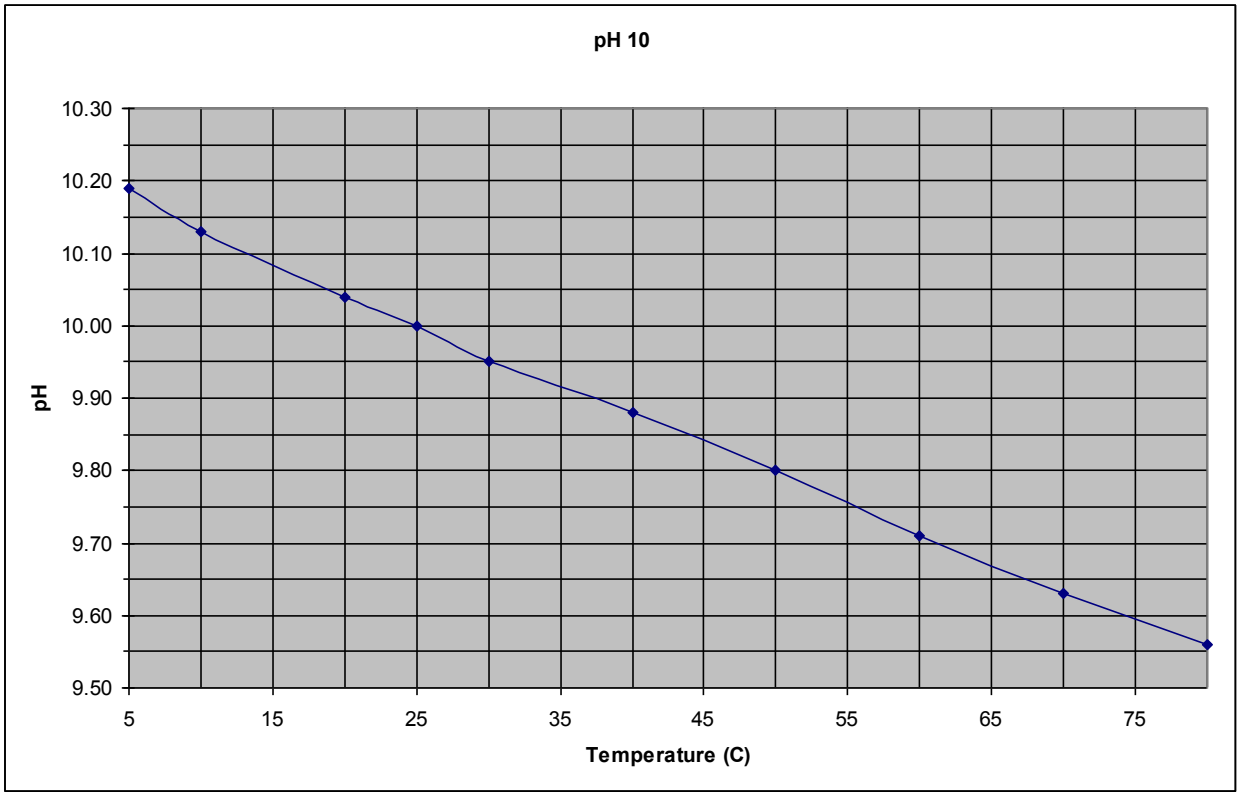

Figure 37. Temperature/pH curve for pH 10 Buffer Solution

14. Type in the exact pH for the 10.00 buffer solution given the current room temperature; press **Enter**. Allow readout to stabilize (approximately one minute).

15. Record the initial or pre-calibration readout; press **Enter** to calibrate.

16. Record the final or calibration readout and press **Enter** again.

17. Rinse probe 2 times with DI or distilled water and once with pH 4.00 buffer solution.

18. Fill calibration cup with 4.00 buffer solution to within a centimeter of the top of the cup.

19. Determine the exact current pH of the 4.00 buffer solution from the table provided by the manufacturer or in ***Figure 38 below*** as in Step 7 above.

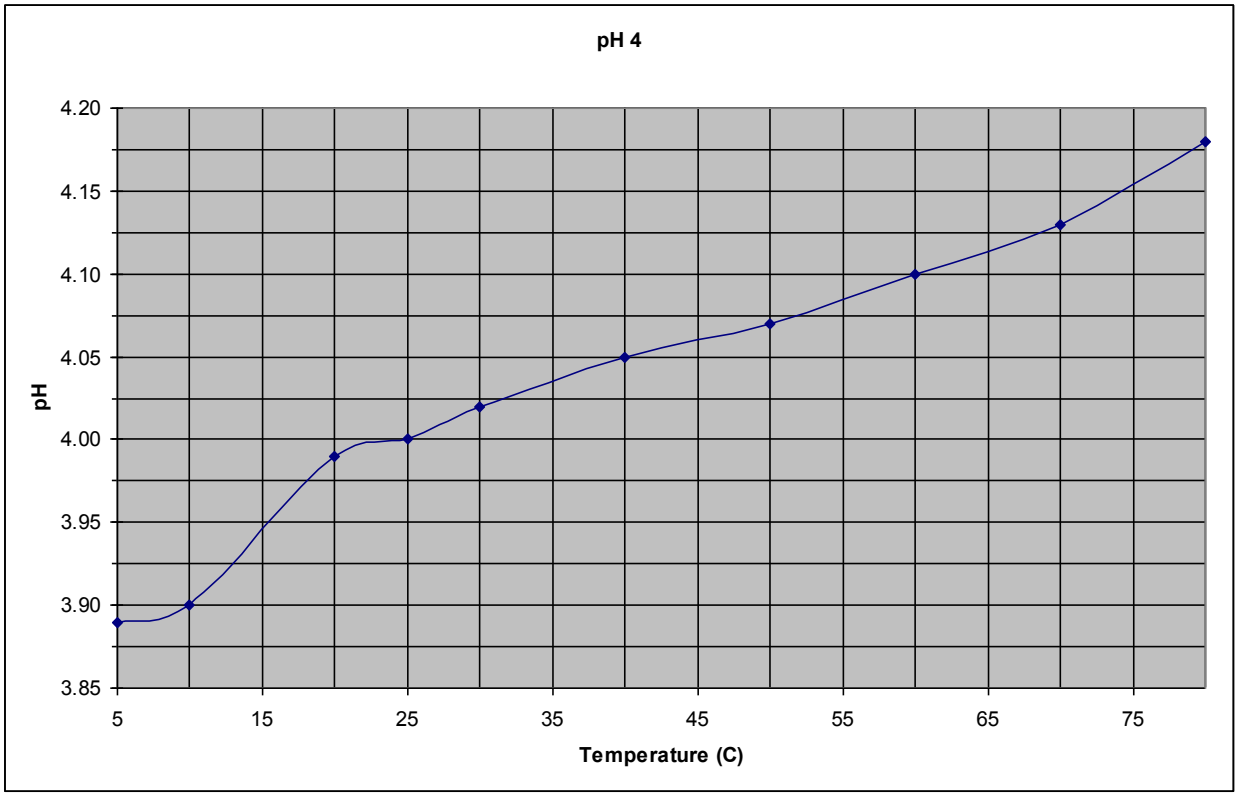

Figure 38. Temperature/pH curve for pH 4 Buffer Solution

20. Type in exact pH for the 4.00 buffer solution given the current room temperature; press **Enter**. Allow reading to stabilize (approximately one minute).

21. Record the initial or pre-calibration readout; press **Enter** to calibrate. Record the final or calibrated readout. Press enter to return to the pH calibration menu.

22. Pour out some of the 4.00 buffer solution from the calibration cup, leaving some behind to keep the air inside the cup moist. Preferably, the sonde should always be stored in some sort of high ionic solution (such as the pH buffer solutions) as this will prevent leaching of ions from the pH probe and prevent degradation to the probe's expected lifespan. Should you spill the buffer out of the calibration cup while in the field, add an *extremely small* amount of stream water (just enough to keep the air inside the cup moist) to the storage cup. THE WATER SHOULD NOT COVER THE PH OR DO PROBE WHEN THE SONDE IS HORIZONTAL. This water should be replaced by buffer solution as soon as possible to prevent the aforementioned degradation of the pH probe.

B) Probe Maintenance and Troubleshooting

Sometimes slow response times or instability with the values (jumping as much as +/- 1.0 unit during calibration or field measurements) are observed with the pH readings. This may be caused by a number of factors and may or may not be indicative of a bad probe.

One consideration is the age of the probe. YSI's pH probes have an expected use lifetime of about 18 months. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe is 18 months or older, then it is likely that it has passed its expected lifetime and it may simply be too old to give proper calibration and readings.

Another factor to consider is the temperature probe. The calculation of the pH by the sonde is a temperature dependent calculation. If the temperature probe is malfunctioning, then it may appear as if the pH probe isn't working right. Be sure to check the temperature to see if it returning a reasonable value. If it is not, then the temperature/conductivity probe may need to be replaced.

Water or sealant grease can also get in the connector when replacing a probe and can cause malfunctions and erratic readings. When replacing a pH probe, dry off the probe and sonde as much as you can before removing the probe to make sure water doesn't enter the fitting. Also, remove the pH probe with the sonde upside down so that water cannot run into the connections. Once removed, look inside the connector end of the probe and sonde to see if there is any water or grease in the fitting. If so, remove it with a can of compressed air and/or a paper towel. The important thing is to dry it out as much as possible. If there is excessive grease, then try to remove it with a towel. If the grease cannot be removed, an YSI maintenance expert may need to use a solvent for to break up the grease. Once dry, reconnect the probe using very little grease around the upper O-ring near the threads. A very thin coat making the O-ring look wet is sufficient for a proper seal.

Cleaning is required when response becomes slow or when deposits build up on the surfaces. To clean the glass bulb, remove the probe and use a soft cloth or tissue to wipe foreign material from the glass bulb and platinum button. Then use a moisten cotton swab to GENTLY remove any material blocking the reference electrode junction. DO NOT WEDGE THE SWAB TIP BETWEEN THE GUARD AND THE GLASS SENSOR.

If response is still slow, soak the probe 10-15 minutes in clean water containing dishwashing liquid. Then wipe the probes gently with a cotton swab moistened with

the cleaning liquid. Rinse in clean water, wipe once more with a clean swab and rinse again.

If response times continue to be slow, the probe may be cleaned in a 1:1 chlorine bleach solution for 1 hour. YSI recommends this procedure ever 6 – 12 months if the probe does not work well. This is usually as result of extreme conditions in which fowling of the probe is more probable.

Finally, if the probe still does not respond well, it may be soaked in one molar HCl for 30-60 minutes. THIS SHOULD BE USED AS A LAST RESORT METHOD ONLY. REFER TO THE YSI OPERATING AND MAINTENANCE MANUAL FOR DETAILS ON THESE PROCEDURES OR CONSULT AN YSI REPRESENTIVE.

C) pH Probe Diagnostic (Nernst Equation Calculation)

The pH probe on an YSI sonde operates using the Nernst Equation (***see Figure 39 below***). Simply put, a line running from 4 to 7 (or 7 to 10) pH on the x-axis should increase 180 mV (or 60 mV/pH unit) from 7 to 4 or (decrease 180 mV from 7 to 10) pH on the y-axis as in the illustration below. If this slope flattens, the pH probe will lose resolution. This slope is a result of the probe condition as well as the quality of the calibration. A function called pH mV may be used to check this slope.

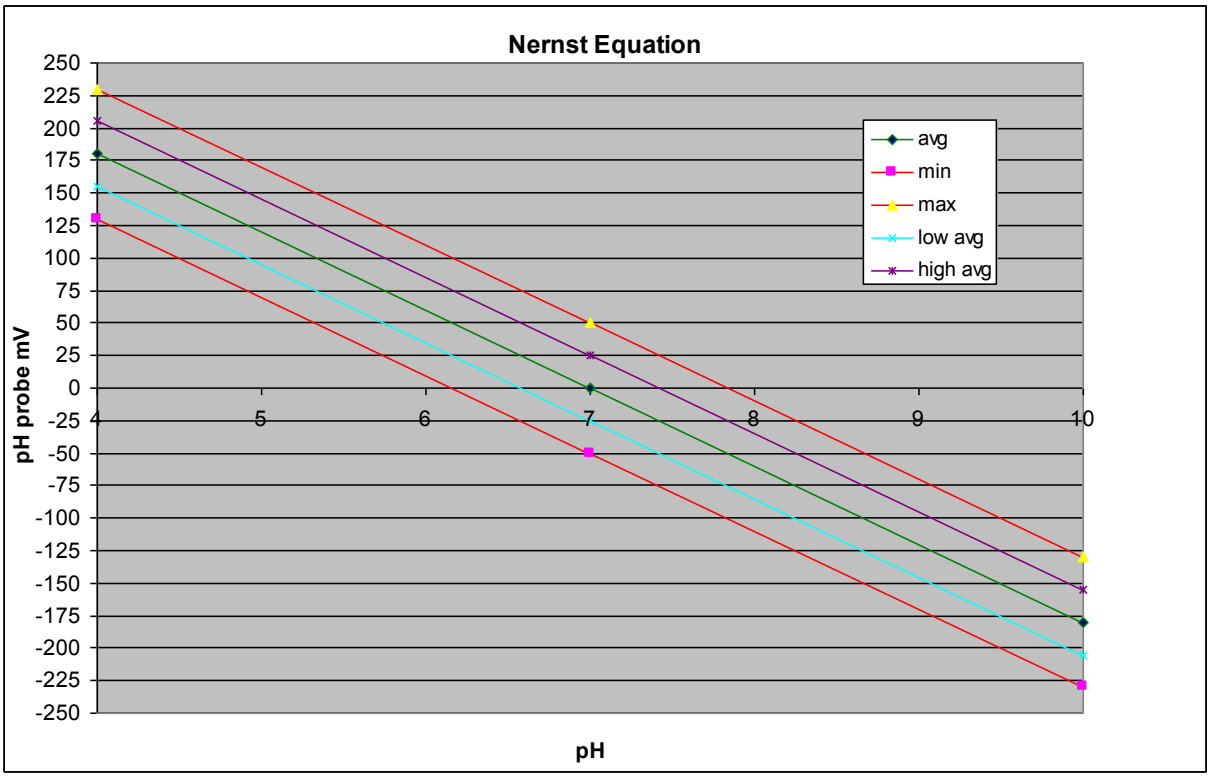

Figure 39. The Nernst Equation

The Nernst Equation may be calculated by following these steps:

1. Press **Esc** to get the Main Menu.

2. Use the **Up** or **Down** keys to scroll to Report and press Enter.

3. Scroll down and select “pH mV” and press **Enter**. When this is done, the symbol to the left of “pH mV” should change from an empty to a black circle.

4. Press **Esc** twice to get the 650 Main Menu. Scroll up to “Sonde Run” and press Enter.

5. A new, second parameter called “pH mV” should be visible on the screen that reads well beyond 14 and may be positive or negative.

6. Use the rinse procedures from the pH calibration above to introduce the 7.00 buffer solution to the probe. Write down the second “pH” reading for the 7.00 buffer solution. It should be between –30 and 30, but may be slightly more (-50 to 50). The reading should stabilize within the aforementioned values in less than 30 seconds.

7. Repeat the rinse procedures for either 4.00 or 10.00 buffer solution. For either solution, the new reading should have a difference of 180 from the initial 7.00 buffer solution reading.

   For example, if an initial reading at 7.00 was -20.0, then the second reading at 4.00 should be around 160 (+/- 30)or, if using 10.00, the second reading should be around –200 (+/- 30).

8. If the probe reads in this range within 30 seconds, then the probe is ok and simply repeat this procedure to turn off the pH mV function (the black circle will change back into a **–**). If the probe reads outside this range or takes longer than 30 seconds, two things may be wrong. First, the calibration may be off and the calibration procedure should be repeated to check for this. Unfortunately, if recalibration does not correct the problem, this is an indication that the KCl solution inside the probe is contaminated with water and the whole probe will need to be replaced. ***See YSI Sonde Storage on page 121 for prevention of contamination of the KCl solution inside the pH probe.***

D) pH Probe Accuracy

The pH probe accuracy is +/- 0.2 pH units (*i.e.,* 6.8-7.2 is an acceptable reading for 7.0 buffer solution.

5) Temperature

Temperature Probe Accuracy

The Temperature probe accuracy is +/- 0.15^0 C.

YSI Sonde Storage

The pH probe on an YSI sonde operates using a polypropylene wick from the water (or sampling) side to a concentrated KCl side sealed inside the probe. To increase the life of a probe, proper storage of the probe must be implemented.

If the probe is being stored for a **short period of time**, place only a minute amount of water (1/8th of an inch is probably too much) in the cup for storage making sure that no water will inundate the pH probe. The other probes (*e.g.,* the DO probe) require only moist air to maintain proper function. A small damp sponge inside the cup would be adequate for such storage.

For **long-term storage** (*e.g.,* over winter), it is recommended that the cup be filled with a concentrated KCl solution. This will lengthen the life of the probe and help maintain the concentration of KCl inside of the pH probe. TO OBTAIN THE CONCENTRATION OF KCL SOLUTION, CONSULT THE YSI OPERATING MANUAL OR AN YSI REPRESENTATIVE.

Hydrolab Quanta G Calibration

These directions are very similar to the older Scout 2 Hydrolab directions. However, individuals with no prior experience should calibrate with the assistance of an experienced user.

1) Quanta G Display Unit

The Quanta G display unit uses a series of escapable menus in conjunction with several keys in the calibration process. Become familiar with the Enter key (which looks like a left arrow with a right angle), escape (Esc ∞), on/off (O | I), and arrow keys as these will be the most often used.

Maintenance of Quanta G Display

The Quanta G runs on 3 C batteries. Replace the C batteries as required. The Quanta G System provides at least 15 hours of continuous operation on one set of new batteries. A Battery Low icon will show the battery status.

2) Dissolved Oxygen

A) DO Probe Calibration

1. Remove calibration cup from probe and dry the membrane by blotting with a soft cloth or tissue. Check the membrane for wrinkles, tears, bubbles, dirt, etc. and replace membrane, if necessary.

2. Attach calibration cup to the Quanta and fill cup with room temperature tap water until the water surface is just level with O-ring on the D.O. probe. Do not pour water on the membrane. If the membrane gets wet, blot dry with a soft cloth or tissue.

3. Cover the calibration cup loosely using the black calibration cup cover placed upside down on the calibration cup.

4. Let the unit sit for about 10 minutes so that the air inside the cup will saturate with water.

5. Turn on the Quanta G using the **O | I** key and allow the D.O. reading to stabilize. If the circulator is on, press the **Esc** ∞ key to toggle the circulator off so that it doesn't splash the water in the cup onto the membrane. Record the initial or pre-calibration readings (mg/L) on the Sonde Calibration Log Sheet. Also record the initial readout for temperature.

6. Press the **enter** key to toggle to the next screen and record the initial or pre-calibration % DO saturation on the Sonde Calibration Log Sheet.

7. After power-up the Display's "Screen" icon in the lower center of the screen is blinking. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "DO" to blink and the press the **enter** key.

8. Determine the barometric pressure for entry as the calibration standard and record on the Sonde Calibration Log Sheet. *Use the local barometric pressure. Many local weather bureaus correct the barometric pressure to sea level. Consult the operating manual for formulas to convert from sea level barometric pressure to local barometric pressure.*

9. Press the **arrow** keys to raise or lower the barometric pressure to match the calibration standard.

10. Press the **enter** key to finish calibration of Dissolved Oxygen. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen.

11. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration D.O. readings on the Sonde Calibration Log Sheet. Press the **enter** key to toggle to the next screen and record the final or post-calibration % DO saturation on the Sonde Calibration Log Sheet.

B) DO Probe Maintenance

If the D.O. will not calibrate, the membrane may be torn, wrinkled, dirty, damaged, or a bubble may be trapped in the probe. The membrane should be replaced whenever these conditions are observed. Frequent replacement of membranes can also lengthen the life of the probe.

To change the membrane, remove the calibration cup. Remove the O-ring that holds the membrane on the probe. Shake out the old electrolyte solution, rinse the probe with electrolyte solution, and refill with fresh electrolyte until a positive meniscus rises above the probe surface. Make sure there are no bubbles in the probe. Install the new membrane (don't stretch the membrane while doing this), and replace the O-ring. If possible allow the probe to soak overnight in tap water to acclimate to its new shape.

C) DO Probe Accuracy

The DO probe accuracy is +/- 0.2 mg/L (or ppm) O_2 at ≤ 20 mg/L or +/- 0.6 mg/L (or ppm) O_2 at >20 mg/L.

3) Conductivity

A) Conductivity Probe Calibration

1. Remove the lid on the calibration cup and rinse the probe 3 times with DI or distilled water.

2. Rinse the probe 2 times with a small amount of **fresh** conductivity standard in the **1000-5000** microSiemens or μS (also known as micromhos or μmhos/cm) range. The exact concentration of the standard will be written on the bottle. The concentration of solution used should be dictated by the conductivities expected to be encountered during the use of the instrument during the week.

3. Fill cup with conductivity standard to within a centimeter of the top of the cup. Make sure that there are no bubbles in the measurement cell of the specific conductance sensor. Wait for the readings to stabilize. Record the concentration of calibration standard used and the initial or pre-calibration specific conductance readings on the Sonde Calibration Log Sheet.

4. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "SpC" to blink and the press the **enter** key.

5. Press the **arrow** keys to raise or lower the specific conductance to match the calibration standard in mS/cm.

6. Press the **enter** key to finish calibration of specific conductance. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen.

7. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration specific conductance readings on the Sonde Calibration Log Sheet.

8. Rinse at least 3 times with DI or distilled water.

9. Conduct a **Low-End Specific Conductance Check** using either DI or distilled water (expected conductance =< 5 microSiemens). This Low-End Specific Conductance Check should be conducted at least once a week during calibration. Indicate the solution used and the Low-End conductivity solution reading on the Sonde Calibration Log Sheet.

10. At least once a month, conduct a **Mid-Range Specific Conductance Check** using either a known 500 or 1000 microSiemens solution, depending on the solution used to calibrate the conductivity probe. Indicate the conductivity of the solution used and the Mid-Range conductivity solution reading on the Sonde Calibration Log Sheet.

B) Conductivity Probe Maintenance

Clean the oval measurement cell on the specific conductance sensor with a small, non-abrasive brush or cotton swab. Soap or rubbing alcohol may be used to remove grease, oil, or biological material. Rinse with water.

C) Conductivity Probe Accuracy

The Conductivity probe accuracy is +/- 1% +/- 1 μS/cm. For example, a solution that is 1000 MicroSiemens, the range would be 1000 x 0.01 +/- 1 MicroSiemen or 10 +/- 1 MicroSiemen.

4) pH

The pH probes on a Hydrolab sonde only offer one type of calibration: Two-Point.

A) pH Probe Calibration (a Two-Point calibration)

1. Rinse the probe 3 times with DI or distilled water.

2. Rinse the probe 2 times with a small amount of the 7.0 pH standard.

3. Fill cup with 7.0 pH standard to within a centimeter of the top of the cup. Wait for the readings to stabilize. Record initial or pre-calibration specific conductance readings on the Sonde Calibration Log Sheet.

4. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "pH" to blink and the press the **enter** key.

5. Press the **arrow** keys to raise or lower the pH to match the calibration standard for the given room temperature (***See Figure 36 on page 115 under YSI 600XL Sonde/650 MDS Display Unit Calibration pH***).

6. Press the **enter** key to finish calibration of pH. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen

7. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration pH readings on the Sonde Calibration Log Sheet.

8. Repeat steps 1-7 for the second pH standard. This pH standard will depend on the types of streams that will be encountered. Use the 4.0 pH buffer if mainly acid streams will be encountered and use the 10.0 pH buffer if mainly alkaline streams will be encountered. The calibration standards exact pH at the given temperature can be found in ***Figure 37 and Figure 38*** (***See pages 116 & 117 under YSI 600XL Sonde/650 MDS Display Unit Calibration pH***).

9. When finished with the second pH standard, add a *very* small amount of tap water (just enough to keep the air inside the cup moist) to the storage cup. THE STORAGE WATER SHOULD NOT COVER THE PH OR DO PROBE WHEN THE SONDE IS HORIZONTAL.

B) pH Probe Maintenance

Two electrodes are used to measure pH: a glass pH probe and a reference electrode enclosed in a reference sleeve. If the response time for pH seems slow, refer the owner's manual for cleaning instructions.

Glass pH probe: Little maintenance is required. Check the tip of the probe to make sure the glass is not broken or dirty. If the pH sensor is obviously coated with oil, sediment, or biological growth, clean the glass with a very clean, soft, non-scratching cloth wet with rubbing alcohol (a cotton ball will do). Rinse with tap water.

Reference electrode: Gently pull the reference sleeve away from the probe. The reference sleeve is the black tube with a porous Teflon Reference Junction attached. Discard the old electrolyte from the reference sleeve. Refill the sleeve to the top with reference electrolyte. With the probe pointed toward the floor, push the full reference sleeve back onto its mount until the sleeve has just covered the first O-ring located on the mount (just behind the silver electrode). Turn the probe so that the sensors point toward the ceiling and push the sleeve the rest of the way onto its mount. Rinse with tap water. The porous Teflon Reference Junction is the most important part of the pH performance. Make sure it is clean and passes electrolyte readily. If not, replace it. When seating the reference sleeve, trapped air and excess electrolyte is purged. This purging flushes and cleans the porous Teflon Reference Junction.

C) Conductivity Probe Accuracy

The pH probe accuracy is +/- 0.2 pH units (*i.e.,* 6.8-7.2 is an acceptable reading for 7.0 buffer solution.

5) Temperature

Temperature Probe Accuracy

The Temperature probe accuracy is +/- 0.2^0 C.

Quanta G Probe Storage

When not in use, the H20 should be stored with the storage cup containing about ½ inch of tap water. In an emergency, the cup can be filled with ½ inch of clean creek water. The creek water should be replaced with tap water when you return to the lab. The pH reference electrode should also be stored in saturated KCl solution under the plastic cap.

Part 2. Field Procedures

The readings from a water quality sonde are often referred to as instantaneous readings as they are taking immediately and directly from the water column.

While weekly calibration should be adequate to take care of the majority of the probes, the DO probe should be calibrated daily. In some cases, when travel to sites are greatly varying elevations, DO should be calibrated in each new elevation.

In the case of the Hydrolab Quanta, the pH should be recalibrated if the pH regime of the stream changes (*e.g.,* the Quanta was calibrated for 4.0 to 7.0 and the streams you are sampling are above 7.0 pH).

Setting up the Water Quality Sample Site

1. Attempt to locate a good sampling location with adequate depth and flow near mid-stream. If mid-stream is not available due to high flows or deep water, you may take deploy the sonde from the bank if you are sure that there is no plumes from pollution sources or tributaries that may be flowing along either bank. Additionally, if the cord of your sonde is long enough, you could attempt to deploy the sonde from a bridge. Another alternative is to deploy the sonde into a proxy like a bucket or sample tube that was lowered off of a bridge to collect water. In any case, be sure to document where and how you sampled on the habitat form. **IF YOU ARE COLLECTING WATER FOR ANALYSIS AT A LAB, DIRECTLY FROM THE STREAM, YOU MUST PLACE THE SONDE IN THE SAME FLOW VECTOR AS THE WATER SAMPLE COLLECTION.**

2. Remove the calibration cup from the end of the sonde, screw on the deployment guard, and deploy the sonde into the water column. **Be sure to not disturb the substrate above this point until all water data collection is completed.**

   Note: When deploying a sonde into the water, give it a little tap or shake once submerged. This will help dislodge any air bubbles inside the conductivity probe that will bias a reading. Make sure that all probes are submerged adequately.

3. Once fully submerged in the water turn the unit on. For YSI, turn on the unit with the power key and press **Enter** twice. For the Hydrolab Quanta, turn on the unit using the **O | I** key. Press the **Esc** ∞ key to toggle the circulator on and off if necessary.

4. Let the readings stabilize for at least ten minutes. Note that it has been observed that the pH readings take as long as 20-30 minutes to stabilize under extremely low temperature or low conductivity situations. In addition, the age of the sonde/probes also can play a role in how long it takes for the readings to stabilize. This time could be used to fill out parts of the habitat form, collect water samples, or check on the GPS coordinates.

5. Record the readings onto the habitat form and turn the sonde off. Take off the deployment guard and replace the calibration cup. Always make sure sand and other particles are kept clear of the threads on the sampling weight, cap, storage cup, and sonde itself. These threads are plastic and will strip if sand is caught in the treads while screwing these parts on and off.

6. Store the sonde securely for future use. When storing the sonde between sites or sampling events, only a small amount of 4.0 pH buffer inside the cup is necessary to keep the air (and membranes) moist. If the pH buffer is spilled at the site, you can get away with a few drops of water inside the cup until you can replace it back at a vehicle or the lab. DO NOT STORE THE SONDE WITH A FULL CUP OF WATER, AS THIS WILL LESSEN THE LIFE OF THE pH PROBE.

Tips for usage of YSI probes in the field:

- If taking readings from an intermediate water container (*e.g.,* a bucket), make sure to keep the hole on the conductivity probe away from the edges of the container as this may cause stray signals from the probe and result in an inaccurate reading. Also attempt to keep the sample as sealed and isolated as possible to maintain the temperature and DO concentrations. It also may be necessary to swirl the sonde around to keep the water circulating.
- If a DO probe is suspected of being out of calibration, check the DO charge reading as well as the % air saturation. If the air saturation is not within an expected range for your current elevation, recalibration at that elevation may be necessary. It is also possible that the internal barometer needs recalibration (a manufacturer repair).

Sonde Quality Assurance/Quality Control

Before use, each sonde and probe should be examined for wear (*e.g.,* breakage, air bubbles, membrane tears or wrinkles) and adjustments should be made as required.

Sonde Calibration Log Sheets are used for any calibration or maintenance performed on an instrument and entered into the database weekly and examined for patterns that indicate potential or imminent probe failure. Any instrument failing to meet calibration requirements is repaired in house or shipped to the manufacturer. Meters are calibrated weekly, prior to sampling, and are recalibrated in the field, if conditions warrant. For example, if a Hydrolab has been calibrated for pH using the 7 and 10 buffers, recalibration is performed if a stream pH of 3 is encountered. Note that an YSI sonde has a 3-point pH calibration procedure available that is conducted in the lab since streams in the acidic and alkaline ranges are often both encountered during the course of a week of sampling. D. O. is calibrated daily. In addition, all sondes and probes are cross-checked against each other monthly for accuracy and stabilization speed.

Each meter has an identification number, which is recorded on the habitat assessment sheet each time the meter is used. Should any instrument fail to calibrate, readings taken prior to the failed calibration will be examined for reliability and accuracy. Documentation of the instrument used at each site will help to keep data loss to a minimum. All repairs to sondes and probes are documented in a repair log including the serial numbers and manufacture dates of any replacement probes.

Duplication of sonde data is only possible if two different sondes are present during the sampling event. If this is the case, then the sampler should be all means document the duplication of sonde data.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. A hands-on session concerning the calibration, maintenance, and collection of water quality sonde data is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in using water quality sondes will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to use the sondes solo. This document is also provided to all program personnel for review and use in the field.

Section B. Water Quality Sample Collection and Preservation

The water quality monitoring is the centerpiece of the Watershed Assessment Branch's (WAB) efforts to assess streams. It is extremely important that all of these methods are followed to maintain comparability between samplers and sampling events.

Materials and Reagents

1. Analysis Request Form with Chain-of-Custody (COC) - for sample identification and tracking of samples from the field to lab and results from the lab back to us.
2. Water Quality Sample Labels-featuring the unique WQ Sample ID for each distinct water sample.
3. Waterproof pen or sharpie - for labeling sample bottles.
4. Sterile Fecal bottles with Sodium thiosulfate tablet - for collecting bacteria samples.
5. Plastic Containers (*e.g.*, cubitainers with Lids) - for collecting other water quality samples, except phenols.
6. Cooler - for sample preservation.
7. Wet Ice - for sample preservation.
8. Fixatives (nitric acid, sulfuric acid, and sodium hydroxide) - for sample preservation.
9. Waterproof plastic bags or other suitable container - for holding bacteria sample bottles during transport.
10. Filtration Apparatus (either Peristaltic or Vacuum type) – for sample preservation.

Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have

sharp objects embedded in the substrate (*e.g.,* glass, metal, wire, etc.). They should also be worn during sample preservation or at any time while handling the fixatives, which are concentrated acids. Bottles containing fixatives should be stably seated inside a lidded container to prevent breakage and leakage.

WARNING!! SOME FIXATIVES ARE CORROSIVE AND MAY EMIT TOXIC FUMES. BE SURE TO USE THE APPROPRIATE SAFETY GEAR AND PRESEVE SAMPLES IN A WELL VENTALIATED AREA. DO NOT FIX SAMPLES IN THE VEHICLE, AS ACCIDENTAL SPILLS CAN AND WILL OCCUR.

Do not place liquid acid or base into sample bottles prior to sample collection. Always add fixatives to sample. **NEVER ADD SAMPLE WATER TO LIQUID ACIDS OR BASES, AS A STRONG CHEMICAL REACTION CAN OCCUR.**

Part 1. Procedures for Collecting Water Quality Samples

Labeling Sample Containers

Label each sample container with a sharpie. The following information must be included: Agency Name (WVDEP), Project Name (*e.g.,* WAB, TMDL, Deploy, AWQN, LTMS, Lakes, etc.), WQ Sample ID (this is a unique number for each water sample that is taken from the Water Quality Sample Labels), Stream Name, AN-code and Mile Point, Date/Time (Military) collected, Random # (if applicable) and type of fixative/preservation used. In addition, distinct sub-sample descriptions should be indicated somewhere on the form (*e.g.,* Top vs. Bottom, Left Bank vs. Right Bank, etc.). Duplicate or replicate samples should be distinguished by a Dup #1 vs. Dup #2 or Rep #1 vs. Rep #2. It is recommended that some additional identifying mark be put on the lids as the sides of the containers can be abraded pretty easy and lose their labels. This additional identifying mark can be the WQ Sample ID, Random #, or something as simple as the time of collection.

NOTE: The Water Quality Sample Labels do not stick well to any surface that is not smooth. Therefore, if a fecal sample is being collected, use the label on the fecal bottle. If no fecal sample (or other sample that has a container with a smooth surface) is being collected, then copy the WQ Sample ID to other containers and discard the unused label.

Direct Dip/Grab Method

1. At the selected water quality sampling location, (X-site for random sites), attempt to locate a good sampling location with adequate depth and flow near mid-stream. If mid-stream is not available due to high flows or deep water, you may take the sample from the bank if you are sure that there is no plumes from pollution sources or tributaries that may be flowing along either bank. Be sure to document where you

sampled on the habitat form. **Be sure to not disturb the substrate above this point until all water sampling is completed.**

2. Place the water quality sonde downstream in the same flow vector as your sample point and turn it on so that it can begin to take readings (***see Section A. Part 2. Field Procedures starting on page 126 for more information on how to use the water quality sonde in the field***).

3. Collect the water samples upstream as follows:
   A. Fecal Coliform Sample:
   - Use pre-sterilized bottle with Sodium thiosulfate tablet. Keep the bottle closed until you are ready to collect the sample.
   - Open bottle and handle carefully to avoid contamination. DO NOT TOUCH THE INSIDE OF THE LID OR BOTTLE.
   - Using a quick dipping motion, submerge and fill the bottle to the 100 ml mark. DO NOT RINSE OR REFILL THE BOTTLE. If the bottle is too full, slowly pour a little out. *The head-space is necessary in a fecal sample to provide oxygen to the bacteria until the sample can be analyzed.*
   - Place cap tightly on bottle and secure cap lock.

   B. Other Water Samples (*e.g.*, cubitainer Samples):
   - All remaining water quality samples are collected in containers (*e.g.*, cubitainers) provided by the analytical lab (per the state analytical contract). ***If using cubitainers or other collapsible container, do not blow into the containers to open them if at all possible. This is especially true for the Nutrient sample container (preserved with H_2SO_4).***

     Note: If water depth is limiting the ability to submerge the container without stirring up sediment and organic debris, you may use a fresh, unused fecal bottle that has been thoroughly rinsed with stream water (more than three times to remove any Sodium thiosulfate residue) to fill the container. After use, discard the fecal bottle.

   - Rinse the sample container **three times** at least **one-half full** with sample water. During the rinse, secure the lid on the containers, shake for 5 seconds, and then empty.
   - Submerge and fill the containers with sample water and expunge as much of the airspace as possible.
   - Make sure to limit pieces of organic matter in the sample container as much as possible as these can cause contamination.
   - When sealing the container, remove as much air as possible should be expunged from the sample container to avoid contamination (*i.e.,* no head-space).

Remember: Take water samples at lower end of reach for Non- Random targeted sites. Take water samples at X-site for random sites regardless of the location of the lower end of 100 m assessment reach.

Indirect Methods

In some cases, water levels (high or low) or strong flows will not permit direct water sampling. In these cases, it is necessary to use special equipment in order to get the sample. The use of such equipment is explained in ***Chapter X. Section A. Part 2. Bridge Crane Method starting on page 254 and Chapter XI. Section D. Part 3. Van Dorn Sampler Method for Depth Profiles on page 306.*** In the case of low flows, the use of a surrogate container (*e.g.*, a clean, unused fecal sample container) may be necessary to fill the sample container. When doing this, make sure to thoroughly rinse out any of the Sodium Thiosulfate that may be in the Fecal container before filling the sample containers.

Part 2. Sample Preservation (Filtration, Fixation, & Holding)

Preserve the sample as indicated on the Analysis Request Form. ***See Figure 40 on page 141 for an example of an Analysis Request Form with COC***. **Preservation must occur within 15 minutes of sample collection, even if you must pack bags of wet ice and the filtration equipment in on a 6 mile hike. NO EXCEPTIONS!** ***The preservation methods and holding times are summarized in Table 9 in the Holding section on page 139.***

Samples should be preserved in the following order:

1) Unfixed or Iced (Wet Ice) Samples (*e.g.,* Fecal Coliform, Unfixed container)
2) Filtered Samples (*e.g.,* Dissolved Metals or Nutrients)
3) Fixed Samples (*e.g.,* Total Metals or Nutrients).

The fecal coliform sample should be double bagged before being put on wet ice to prevent accidental contamination of other samples should the sample container become compromised. Do not submerge the fecal sample in ice water! The samples that only need to be cooled on ice (commonly referred to as Unfixed or No Fix) can also be placed on wet ice at this time.

Remember: If Alkalinity is being analyzed, 100% of the air must be expunged from the unfixed containers *to avoid contamination. If nutrients (i.e., Nitrogen or Phosphorus) are being analyzed, avoid water samples with visible organic debris in the container.*

Filtration

A net minimum of 200 mL of filtered sample should be turned in for dissolved metal or nutrient analysis at most labs we deal with.

Protocols for Sample Filtration with Peristaltic Pump/Drill Apparatus (Dissolved Metals & Dissolved Nutrients)

The components of the filtering apparatus are:

1. Peristaltic Pump mounted on Stabilizing Board
2. Power Drill with Pump Adaptor Bit
3. Tygon Tubing
4. Filters (50 mm cellulose acetate membranes with a 0.45 micron pore size); two varieties: Flat Disc or Cartridge.
5. Two sample containers (one for the stream sample and one to receive the filtered water).

It is important to keep the filtering equipment and area around the equipment clean. Try to handle all parts by the exterior components. Fingerprints and other dirt can contaminate samples. The tubing and filters should be kept in their sealed plastic bags until time of use to reduce exposure to dust and other contaminants.

Ideally, the filtering process would occur at streamside by taking the filtered samples (*e.g.,* dissolved metals and dissolved orthophosphate) directly from the water column. However, this is dependent upon there being a flat, streamside surface to work on and no precipitation that could short the drill. If filtering cannot occur directly from the water column, the sample water to be filtered should be collected in a clean container that is rinsed twice with stream water and transported to a suitable area for filtration and preservation within 15 minutes of collection. **This container should be the only one that will be exposed to the Tygon tubing and not reused from site to site. Do not filter from the Total Metals sample container as the insertion of the Tygon tubing may contaminate the sample.**

Procedure:

1. Assemble the filtration unit:
   - Place the drill upside down on stabilizing board and carefully insert the bit into the peristaltic pump.
     - The bit may need to be rotated slightly in order to line up with the receiving shaft and engage fully.
   - Place the unfiltered stream sample container near the pump and remove the cap.

- Open the pump clamp by lifting the lever.
- Without directly touching the tubing, open the sealed tubing bag and remove about 8 inches of tubing. Place this end into the unfiltered stream sample container. The rest of the tubing can now be manipulated directly with the hands, but avoid touching the other end of the tubing if at all possible. Thread the tubing through the pump and close clamp.
  - If filtering directly from stream, the tubing can be touched with the hands.
  - Place the stream end of the tubing so that sediment is not being collected from streambed.
- Attach the filter to one end of tubing
  - Handle filter by edges only, with the pressure valve facing toward the pump and stream sample. The cartridge filters should have an arrow indicating the direction of flow.
  - Make sure not to touch the end of the filter that will be discharging into the dissolved sample container.

2. Flush the filter and tubing briefly with sample water by engaging drill slowly for several seconds.
   - Do not collect the flushed water in the filtered container. Discard elsewhere.

3. Rinse the filtered container:
   - Hold the filter at an angle above the mouth of the receiving (filtered water) container at the point of where the tubing is attached.
     - This will allow the user to feel if pressure is building up too quickly in the tube and prevent the tube from explosively detaching from the filter and potentially contaminating the filtered sample. **Do not hold the tube too tightly as this could also cause leakage around the attachment point.**
   - Engage the drill slowly and fill the receiving container with about 50 mL of water. **DO NOT OPEN THE DRILL FULL THROTTLE AS IT WILL RUPTURE THE FILTER AND CONTAMINATE THE SAMPLE!!!**
   - Cap the container, shake vigorously and discard filtrate.
   - Repeat.

4. Filtering the sample:
   - Engage the drill and fill the receiving container with at least 200 mL, unless otherwise directed. On one liter cubitainers, this location is near the first character on the long diagonal bar on the side of the cubitainer.
     - Use slow drill speeds (never full throttle) to filter the sample, especially when approaching the desired sample about. This method is supposed to be cleaner, not necessarily faster.

- If you are close to being done and the pressure is building to fast in the tubing, try using a pulsation with the drill speed. This will often get you to the end without having to change the filter.

5. Changing filters:
   Sometimes it becomes necessary to change the filter while in the middle of processing a water sample. This is usually due to the filter membrane becoming overwhelmed with small particles of silt, which causes the sample to filter extremely slowly. The filter can also become clogged with seemingly clear water due to unseen periphyton. This will also cause the filter to be changed. If the field personnel feel that it is necessary to change the filter to achieve the minimum amount of sample necessary, the following steps should be taken:
   - Cap the receiving container, relieve the pressure in the tube by reversing the drill momentarily or unlocking the clamp, and remove the clogged filter from the end of the tubing.
   - Replace with a clean filter as before in Step 1 being careful not to touch ends of filter.
   - Flush the new filter as in Step 2 and resume filtering.

   Repeat these steps until a sufficient sample is collected. Record on the lab analysis form how many filters were used. This would give the lab an idea about how high the total suspended solids in the sample should be.

6. You must discard and restart the sample if:
   - The filter is cracked or split during use.
   - The filter is dislodged from tubing while filtering and the unfiltered water contaminates the filtered sample during use. This would be typical if this happens explosively.
   - Sediment is collected directly from bottom of stream.

NOTE: The tubing and filters are disposable, and should only be used once. Discard the each filter after one use and discard the tubing after each sample. Obtain a clean set for the next sampling event.

<u>Protocols for Sample Filtration using a Vacuum Pump (Dissolved Metals & Dissolved Nutrients)</u>

The components of the filtering apparatus are:

1. Filter Flask – Receptacle for the filtered sample
2. Filter Funnel – Consists of two parts: A cup to hold the unfiltered sample and the funnel itself.
3. Filters – Cellulose Nitrate membranes with a 0.45 micron pore size.
4. Vacuum Pump – A variety of hand operated pumps are available.

It is important to keep the filtering apparatus clean. Try to handle all parts by the exterior components or by the stopper. Fingerprints and other dirt can contaminate samples. The Filter Funnel & Filter Flask should be stored in a Zip Lock bag or other container (even when driving from one site to another) to reduce exposure to dust and other contaminants.

Procedure:

The water for the filtered sample must be taken from a portion of the total metals sample.

1. Rinse off the filter apparatus (cup, funnel and flask) with DI or distilled water.
   - Be careful not to get water into the nipple on the flask.
   - Rinse each part separately. Do let rinse water from cup drip into either the funnel or flask and do not let rinse water from the funnel drip into the flask.

2. Assemble the filtration unit:
   - Attach the funnel to the flask.
   - Place a filter on the funnel.
     - Handle the filter by the edges only.
     - Make sure the filter is centered on the funnel's screen.
   - Attach cup, be sure to get a good seal.

3. Initial Rinse:
   - Pour a small amount of sample into cup.
   - Filter sample, making sure all the water has passed through.
   - Depressurize the pump.
   - Wipe drips from exterior of cup & funnel and remove from flask without disassembling cup from funnel.
   - Rinse the flask with a swirling motion and discard filtrate (be careful to avoid getting filtrate in the flask nipple).

4. Filtering the sample:
   - Place cup & funnel assembly back into flask.
   - Pour a larger amount of the sample into the cup.
     - If water is turbid, use small amounts; filter may clog and need to be changed.
     - Do not put too much sample into the cup since this may exceed the capacity of the flask, causing water to be sucked into the pump.
     - Wipe off any spills outside of the cup.
   - Filter sample using full strokes on the pump.
   - Depressurize pump after sample has been filtered and before changing filters.

5. Changing filters:

Sometimes it becomes necessary to change the filter while in the middle of processing a water sample. This is usually due to the filter membrane becoming overwhelmed with small particles of silt which causes filtering to become extremely slow. If the field personnel feel that it is necessary to change the filter to achieve the minimum amount of sample necessary the following steps should be taken:

- If there is any left, pour off the excess water out of the cup by turning the filter apparatus on its side with the siphon arm up so that no filtered water can escape from the flask or enter the vacuum tube. One should support both the cup and the lower funnel so that the two do not break the magnetic seal and separate.
- Filter off any excess water until the filter is dry.
- Remove the cup from the funnel.
- Holding funnel sideways, remove old filter. Start from the top of the filter and pull downward.
- If there is any question that unfiltered water may have dripped into the funnel or into the flask, assume that the sample has been contaminated and the filtering process must be reinitiated from the beginning.
- Install a fresh filter handling only by edges.
- Replace the cup and continue filtering.
- Repeat these steps until sufficient sample (usually a net of 200ml of sample after rinsing the cubitainer 3 times, but check with lab beforehand). It is also a good idea to put on the lab analysis form how may filters were used if greater than 1. This would give the lab an idea about how high the Total Suspended Solids in the sample should be.

7. You must discard and restart the sample if:
   - Filter is cracked or split during use.
   - Sediment on filter is off-center (no white ring around entire edge).

8. End of week cleaning:
   - Rinse cup, funnel and flask with tap water; wipe off scum.
   - Use a brush to lightly clean the funnel's screen.
   - Rinse cup, funnel and flask thoroughly with DI or distilled water and shake off excess droplets.
   - Place a filter on the funnel's screen and store cup/funnel assembled in a zip lock bag.
   - Rinse only the glass flask with 10% HCl. The plastic portions (funnel and cup) may only be rinsed with DI or distilled water and lightly rubbed with a paper towel.
   - Do not touch inside surfaces of filtration apparatus.

Fixation

As outlined in ***Table 9 on page 139 in the Holding section***, some samples will need to be fixed with acids before being stored. Samples that are preserved with Sulfuric Acid should always be preserved before samples that are preserved with Nitric Acid. This is because the volatile Nitric Acid vapors may contaminate Nutrient samples and give false Nitrogen results. If you do accidentally preserve the Nitric Acid sample first, then move away from that area when fixing the Sulfuric Acid (*e.g.,* the opposite end of the vehicle or 20 feet away).

When fixing a sample with acids, careful consideration must be given to the ambient chemistry of the stream (*i.e.,* pH and conductivity) and volume of sample being preserved. Any given ampoule of acid is designed to preserve 1 liter of normal water (*i.e.,* pH near neutral and normal conductivities (approximately 200 μmhos/cm). If a stream has a low pH and/or low conductivity, one ample of acid may over preserve the sample. Conversely, if a stream has a high pH and/or high conductivity, one ample of acid may not be enough to adequately preserve the sample. A larger volume of sample water would also require more acid; a smaller volume less. Less experienced individuals should use pH test strips in order to gage how much acid to add to adequately fix sample.

Testing a sample with a pH test strip

1) First add a small amount of acid to the sample (maybe half of an ampoule).
2) Seal the sample and shake it to mix in the acid.
3) Open the sample and pour a small amount onto a pH test strip. **Never dip the pH test strips into the sample!**
4) Compare the pH test strip color to the color key on the pH test strip package. The target pH is just below 2.
5) If more acid needs to be added, then add more accordingly. Otherwise, seal the sample and put it on wet ice if necessary.

Holding

With the exception of fecal coliform, all samples should be delivered to the lab within the holding times specified in "Standard Methods for the Examination of Water and Wastewater", 18th Edition and as outlined in ***Table 9 Preservation Methods and Holding Times on the next page***.

The holding time for fecal coliform sample has been expanded by the WAB from 6 hours to 24 hours because the six-hour holding time places severe limitations on the amount of time a crew can spend in the field and the majority of these samples are not collected for enforcement purposes. **However, fecal samples collected for the TMDL**

program may need to comply with the six-hour holding time depending on the specific instructions given for that watershed.

Table 9. Preservation Methods and Holding Times

Parameter	Preservation	Max. Holding Time
Fecal Coliform	Cool <10 °C, 0.0008% Na2S2O3	6 hours. (24 hours for TMDL/WAB samples)
Acidity	Cool ≤4 °C	14 days.
Alkalinity	Cool ≤4 °C	14 days.
Ammonia	Cool ≤4 °C, H2SO4 to pH<2	28 days.
Chloride	None required	28 days.
Kjeldahl (TKN) and Organic N	Cool ≤4 °C, H2SO4 to pH<2	28 days.
Chromium VI	Cool ≤4 °C, pH = 9.3–9.7	28 days.
Mercury (CVAA)	HNO3 to pH<2	28 days.
Mercury (CVAFS)	5 mL/L 12N HCl or 5 mL/L BrCl	90 days.
Total Metals (except Boron, Chromium VI, and Mercury)	HNO3 to pH<2	6 months
Dissolved Metals (except Boron, Chromium VI, and Mercury)	Filtered, HNO3 to pH<2	6 months
Nitrate	Cool ≤4 °C	48 hours.
Nitrite	Cool ≤4 °C	48 hours.
Nitrate-Nitrite (NO2-NO3-N)	Cool ≤4 °C, H2SO4 to pH<2	28 days.
Total Orthophosphate	Cool ≤4 °C	48 hours.
Dissolved Orthophosphate	Filtered, Cool ≤4 °C	Filter within 15 minutes; 48 hours.
Phosphorous, Total	Cool ≤4 °C, H2SO4 to pH<2	28 days.
Total Solids, Total Suspended Solids (TSS), Total Dissolved Solids (TDS)	Cool ≤4 °C	7 days.
Sulfate	Cool ≤4 °C	28 days.

Documentation

Using a black or blue permanent ink pen, fill out an Analysis Request Form for each water sample. If multiple samples are taken at a particular site during the sampling event, you will need a separate form for each distinct sub-sample (*e.g.,* Top, Middle, and Bottom Lake samples; Left Bank, Middle, and Right Bank cross-section samples). In addition, you will need to fill out an Analysis Request Form for each laboratory. For example, if the fecal sample will be delivered to one laboratory and the other samples to another, complete a request form for each lab unique to the parameters that they will be analyzing. The person who actually collected the sample must be the person indicated on the form and the one who signs the chain-of-custody (COC).

A completed Analysis Request Form includes: Project, Laboratory Name, Stream Name, WQ Sample ID, Watershed Name, AN-Code, Random # (if applicable), # of Containers, Sampled By, Filtered By, Sample Type, Acid Lot #s, Field Meter # (*i.e.,* Sonde ID), Date and Time (Military), Field Values, Parameters Requested, # of Filters Used, Type of Filters Used, and Relinquished By. In addition, distinct sub-sample descriptions should be indicated somewhere on the form (*e.g.,* Top vs. Bottom, Left Bank vs. Right Bank, etc.). Duplicate or replicate samples should be distinguished by a Dup #1 vs. Dup #2 or Rep #1 vs. Rep #2.

REMEMBER: Until the Analysis Request Form can be updated, put the WQ Sample ID in the spot labeled Station Number.

After the sample has been turned over to a WVDEP certified analytical laboratory (***see on page 149 for more details about the WVDEP Laboratory Quality Assurance Program***), the Date and Military Time, Received By, and Lab name must be filled out on the COC portion at the bottom of the Analysis Request Form. ***See Figure 40 on the next page for an example of a fully completed Analysis Request Form with COC***. Keep the white copy for WAB records and give the yellow copy to the lab.

WV DEPARTMENT OF ENVIRONMENTAL PROTECTION - WATERSHED BRANCH

General Analysis Request Form, Rev. 03/09

TMDL WAS (AWQN) LAKES RANDOM

Laboratory Name: Biochem Circle Activity

Stream Name: Gauley River Watershed Name: Gauley

AN-Code: KG-(813) Station Number: 45681 Random #: _____ # of Containers 5

Sampled By: KDS Filtered By: KDS

Sample Type: Water X Sediment _____ Other _____ Specified Method: **40 CFR 136**

Acid Lot #, Nitric: 926007 Sulfuric: 928803 HCl: _____ Field Meter #25 Flow Meter #_____

Grab: Date-Time: 7/12/10 1300 Lat: _____ Long: _____

Positioning Method (Circle One) Map GPS GIS

Field Values[1]: Temp. 26.52 pH 7.38 D.O. 6.98 Cond. 129 Flow: _____

Pres.	Analysis	Pres.	Analysis	Pres.	Analysis	Tot	Diss[+]
(3)	Acidity (Hot)	3	Tot. Solids	(5)	Sodium		
(3)	Alkalinity	(3)	Tot. Diss. Solids	(5)	Aluminum	(H)	(H)
(5)	Hardness	(3)	Tot. Susp. Solids	(5)	Cadmium	L	(L)
(3)	Sulfate	(4)	T. Phosphorus-P	5	Chromium	H L	H L
3	Turbidity	4	T. Phosphate	(5)	Copper	L	(L)
(2)	Chloride	3	Tot. Ortho PO_4-P	(5)	Iron	(H)	(H)
3	BOD5	8	Diss. Ortho PO_4-P	(5)	Lead	L	(L)
4	COD	(4)	TKN	5	Magnesium		
4	TOC	(4)	Ammonia-N	(5)	Manganese	(H)	
(7)	Fecal Coli., MF 24 hour holding	4	Unionized NH3	5	Mercury	H	
		4	Org-N	(5)	Nickel	L	(L)
7	Fecal Coli., MF 6 hour holding	3	NO_3-N (Nitrate)	(5)	Zinc	L	(L)
		3	NO_2-N (Nitrite)	5	Calcium	H	
7	E. Coli, Numeric	(4)	NO_2-NO_3-N	(5)	Selenium	(L)	
7	Fecal Strep.			(5)	Arsenic	(L)	L
3	pH (lab)	10	Chlorophyl a	(5)	Silver		(L)
3	Cond. (lab)			5	Potassium		
3	Acidity (Cold)	3	Semi-Vol. Organics	(5)	Boron		
		9	Volitile Organics	(5)	Barium		

Preservation Code

1. None - Determined on-site
2. None
3. Iced immediately
4. H_2SO_4 to pH <2, iced immediately (Phenols in glass container)
5. HNO_3 to pH<2
6. (Cyanide) NaOH to pH>12, iced immediately (0.6 g ascorbic acid used on samples with residual chlorine)
7. Sterile + 0.008% $Na_2S_2O_3$, iced immediately
8. Filtered immediately, iced immediately
9. HCl to pH<2, iced immediately
10. $MgCO_3$ & Ice, _____ml sample
11. Other (Specify)

H=High (e.g. 5ppb) L=Low (e.g. 0.05 ppb)

REMARKS:

1 # of Filters used

Filter type (circle): Disc (Cartridge)

[1]Filtered immediately, nitric acid added to pH <2

Relinquished by:	Date & Time	Received by:	Relinquished by:	Date & Time	Received by:
Kevin Seagle	7/12/10 2200	Lab:			Lab:

Mail Results to: ATTN: Janice Smithson (Lab Instructions: On invoice bill to Organization Unit 9480),
WVDEP, DWWM, Watershed Branch, 601 57th Street SE, Charleston, WV 25304 Phone (304) 926-0499 ex. 1051, Fax. 926-0496

WHITE - Sample Collector Copy CANARY -Laboratory Copy

Figure 40. Example of a fully completed Analysis Request Form with Chain-of-Custody (COC) at bottom

Part 3. Common Water Quality Parameter Suites

Take Hydrolab readings at every site!

Because of the susceptibility of the pH and Conductivity probes to infrequent failure, it is important to have redundant information to corroborate the sonde readings. Therefore, always order Lab pH or Lab Specific Conductivity under either of the two following circumstances:

1) The site you are visiting is a single or infrequent visit site (*e.g.*, Random or Target Sites, LTMS, Lakes) and the instantaneous pH reading (*i.e*., sonde) indicates a violation of the pH WQ standards (*i.e*., <6 or >9)

Or

2) The site you are visiting is a multiple visit site (*e.g.*, TMDL, Deployable, Ambient, Special Projects) and the instantaneous pH reading (*i.e*., sonde) is outside of normal historic ranges as indicated on user maintained pH/Conductivity log sheets. For example if the site has a pH reading of 5.5 and has been visited X times in the past year and normally has a pH between 6.5 and 8.0.

Although Lab pH and Lab Specific Conductance readings are not the same as instantaneous readings due to a lag in analysis time, the lab readings should give us the ability to confirm if there is a failure with the sonde's probes.

The Lab Parameter Suites below listed in order of appearance on the COC in Figure 40 on Page 141 above.

Random & Potential Reference Sites:
- Acidity (Hot), Alkalinity, Sulfate, Chloride, Fecal coli., TSS, TDS, Tot. Phos., TKN, NO_2-NO_3-N, Mg (Tot.), K (Tot.), Na (Tot.), Al (Tot. & Dis.), Cu (Dis.), Fe (Tot. & Dis.), Mn (Tot.), Zn (Dis.), Ca (Tot.), Se (Tot.), Bromide, Be (Tot.). (Note: Order Low Level Detection on Tot. & Dis. Cu, Zn, & Se.)

4 containers (wet iced, HNO_3, filtered HNO_3, & H_2SO_4) & fecal bottle

***Acid Rain Parameters*:**
Take when: 1) pH <6.0 & conductivity is <50, 2) if stream is on the 303(d) list for pH unrelated to mining, or 2) if for any reason you suspect acid rain deposition impacting the stream:
- Acidity (Hot), Alkalinity, Sulfate, Fecal coli., Acidity (Cold), TSS, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn (Tot.), & Ca (Tot.).

3 containers (wet iced, HNO_3, & filtered HNO_3) & fecal bottle

***AMD Parameters*:**
Take when: 1) conductivity alone is >500, 2) pH <6.0 & conductivity is >200, 3) if stream is on the 303(d) list for AMD, or 4) if for any reason you suspect mine drainage:
- Acidity (Hot), Alkalinity, Sulfate, Fecal coli., TSS, TDS, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn (Tot.), & Se (Tot. & Dis.). Take Ammonia-N (NH_3) if it is suspected that Ammonia is being used to treat the stream water.

3 containers (wet iced, HNO_3, & filtered HNO_3) & fecal bottle

***Nutrient Enrichment*:**
Take within 24 hours of a significant rain <u>or</u> when animal waste, straight pipes, STP outfalls, etc., may be impacting the stream:
- Fecal coli, TSS, Tot. Phos., TKN, & NO_2-NO_3-N. Take Ammonia-N (NH_3) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia input.

2 containers (wet iced, H_2SO_4) & fecal bottle

***Large River Algae/Nutrient Enrichment*:**
Take in large rivers that are experiencing exceptionally excessive algal blooms (*e.g.*, Greenbrier River, Tygart River):
- Fecal coli, Alkalinity, TSS, Tot. Phos., TKN, NO_2-NO_3-N, Mg (Tot.), & Ca (Tot.). Take Ammonia-N (NH_3) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia input.

3 containers (wet iced, HNO_3, H_2SO_4) & fecal bottle

***TDS Ions*:**
Take anywhere in Monongahela Basin (Dunkard, Monongahela, West Fork, Tygart, Youghiogheny, & Cheat):
- Alkalinity, Sulfate, Chloride, Fecal coli, TDS, Mg (Tot.), K (Tot.), Na (Tot.), & Ca (Tot.).

2 containers (wet iced & HNO_3) & fecal bottle

***Oil & Gas*:**
Take if oil or gas activities are evident & cond. >200 in absence of other sources like AMD:
- Chloride, & Fecal coli.

1 containers (wet iced) & fecal bottle

***Sediment*:**

Typically only used during WQ only sampling events (*e.g.,* TMDL sampling). Take anywhere where it is suspected that Suspended Solids are contributing solely to the Iron load in the stream bed during precipitations events:

- Fecal coli, TSS, & Fe (Tot.).

2 containers (wet iced & HNO_3) & fecal bottle

Current Water Quality Analyte Method Detection Limits

Analyte	Method Detection Level	Analyte	Method Detection Level
Alkalinity	5 mg/L	Kjeldahl Nitrogen	0.1 mg/L
Aluminum	0.005 mg/L	Lead	0.001 mg/L
Ammonia Nitrogen	0.1 mg/L	Magnesium	0.05 mg/L
Antimony	0.005 mg/L	Manganese	0.005 mg/L
Arsenic	0.005 mg/L	MBAS	0.05 mg/L
Barium	0.005 mg/L	Mercury	0.0001 mg/L
Beryllium	0.001 mg/L	Mercury / Method 1631E	0.5 ng/L
Bicarbonate (Standard Methods)	1 mg/L	Mineral Acidity	1 mg/L
BOD	1 mg/L	Molybdenum	0.005 mg/L
BOD-carbonaceous	1 mg/L	Nickel	0.005 mg/L
Boron	0.02 mg/L	Nitrate-Nitrogen	0.05 mg/L
Bromide	1 mg/L	Nitrite-Nitrate	0.05 mg/L
Bromide Alt. Method	0.1 mg/L	Nitrite-Nitrogen	0.05 mg/L
Cadmium	0.0002 mg/L	Oil-Grease	2 mg/L
Calcium	0.02 mg/L	Organic Nitrogen	0.5 mg/L
Chloride	5 mg/L	Orthophosphate	0.01 mg/L
Chlorophyll A	0.5 mg/L	Percent Solids	1%
Chromium	0.001 mg/L	Phenolics	0.01 mg/L
Cobalt	0.001 mg/L	Potassium	0.05 mg/L
COD	0.5 mg/L	Selenium	0.001 mg/L
Color (ADMI)	10 ADMI value	Silver	0.0002 mg/L
Color (APHA)	5 color units	Sodium	0.05 mg/L
Copper	0.001 mg/L	Specific Conductance	3 uS/cm^2
Cyanide, Amenable	0.005 mg/L	Sulfate	5 mg/L
Cyanide, Free (ASTM)	0.01 mg/L	Sulfide	1 mg/L
Dissolved Organic Carbon	1 mg/L	Suspended Solids (TSS)	3 mg/L
Dissolved Solids (TDS)	1 mg/L	Thallium	0.001 mg/L
Escherichia Coli (Numeric Result)	1 col/100 mL	Tin	0.02 mg/L
Fecal Coliform (MF)	4 col/100 mL	TOC	1 mg/L
Fecal Coliform (MPN)	4 col/100 mL	Tot Petroleum Hydrocarbons GRO/DRO (8015)	0.5 mg/L
Fecal Streptococci	4 col/100 mL	Total Acidity	1 mg/L
Ferrous Iron (Standard Methods)	0.05 mg/L	Total Cyanide	0.005 mg/L
Fluoride	0.2 mg/L	Total Phosphate	0.01 mg/L
Hardness	1 mg/L	Total Phosphorus	0.005 mg/L
Hexavalent Chromium	0.005 mg/L	Total Solids	1 mg/L
Hot Acidity	5 mg/l	Turbidity	1 NTU (higher OK if highly turbid)
Hot Acidity Alt. Method	*	Vanadium	0.005 mg/L
Iron	0.01 mg/L	Volatile Solids	1 mg/L
		Zinc	0.002 mg/L

Table 10. Current Water Quality Analyte MDLs

Water Sample Collection Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with water quality sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of water quality samples is included. In the field, biological sampling teams will consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in collecting water quality will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to collect water quality solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Lot numbers of all preservatives are recorded on the Analysis Request Form for each sample submitted and entered into the database to allow for easy tracking. Sample transfer to the lab shall also be documented using the Chain-of-Custody (COC) portion of the Analysis Request Form.

Duplicate sampling and field blanks must be performed at a minimum of 2.5% of our sites. To assure we meet these requirements, each team list will have a designated duplicate and field blank. The field blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented in ***Chapter XIII. Section A. Field Blanks and Duplicates starting on page 353.***

Field Blanks

Overview

To evaluate sample containers for contamination, each team will prepare field blanks weekly. DI or distilled water is used as the blank "sample". This water should be carried in an unused, well-sealed, one-gallon cubitainer. During the designated sampling event, an extra set of sample containers are prepared as field blanks, one container for each type of preservation method. The blanks are labeled according to the protocols. These containers are filled with the DI or distilled water and are preserved and stored in the same manner as the actual samples. A separate Analysis Request Form with Chain-of-Custody (COC) is completed for the field blanks and the samples are submitted to the laboratory.

Field blanks are simply samples of DI or distilled water that are preserved in the field. The purpose of the field blank is to detect onsite contamination and verify the purity of the sample fixatives.

Obtaining the Field Blank Water

Before leaving the office, obtain the DI or distilled water by collecting it directly from the laboratory supplied containers.

Procedures for obtaining water from the laboratory supplied containers are as follows:
1. Fill up an unused, one-gallon cubitainer with some water (approximately 100 mL).
2. Screw on the lid, shake the rinse water, and dump. Repeat.
3. After two rinses, completely fill up the one-gallon cubitainer, expunge any remaining air, and place in the vehicle to be used in the field as a source for the field blank water.

Field blanks are to be prepared in the field only and not in the laboratory or garage. A stream location is sometimes designated on the sample list for a field blank. If you miss the exact location indicated on the sheet, prepare a field blank at the next location. The reason why field blanks are indicated on your list is to remind you to do it AND to assure that field blanks are prepared at random locations and times.

A field blank will consist of any parameters that are or may be analyzed during the work week. This may include:
- 1 full cubitainer for Unfixed Samples (Chlorides, Hot Acidity, Alkalinity, TSS, Sulfates, Lab pH, Lab Cond., Cold Acidity, Total Orthophosphate, etc.)
- 1 full cubitainer for Sulfuric Acid Preserved Samples (Total Phosphorous, TKN, NO_2-NO_3-N, Unionized NH_3)
- ½ full cubitainer for Nitric Acid Preserved Samples (All Total Metals)

- ½ full cubitainer for Filtered Nitric Acid Preserved Samples (All Dissolved Metals)
- ½ full cubitainer for Filtered Unfixed Samples (Dissolved Orthophosphate)

Do not prepare a field blank for fecal samples, as the DI or distilled water is not sterile.

Field Procedures

1. To prepare a field blank, retrieve your pre-filled one-gallon cubitainer with DI or distilled water from storage in the vehicle.
2. Label an appropriate number of one liter sample containers in a manner that it will appear to be an actual water sample to the lab, but will also be recognizable as a field blank to WAB employees.
3. Fix and handle the samples as you would do for a stream sample by substituting the DI/distilled water in the one-gallon cubitainer for actual stream water (including filtering for dissolved parameters if that was or will be done during the week).
4. After the sample has been submitted to the lab, write "FIELD BLANK" at the top of the DEP copy (white) of the Analysis Request Form with Chain-of-Custody (COC) before turning it in with the other forms.

Duplicate Samples

Both duplicates are collected at the same date and time and literally side by side by different individuals. If the sampling team consists of one person, as is often the case during a TMDL assessment, the duplicate is still performed by the one sampler. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been disturbed by the first duplicate. TMDL replicates are collected at any TMDL site with the full potential of parameters on the TMDL list. TMDL replicate sites are not specifically assigned; however, field crews should not repeatedly duplicate the same site.

Duplication will be limited to the water quality parameters assigned to that site; *e.g.,* if the site is fecal only, just do fecal. Duplicates for lists that have varying water analysis suites should be conducted at sites where the most parameters on the list are collected (if such sites exist on the list) and, if repeated, should be rotated to different sites each sampling event.

Results of the duplicates are compared and any samples not falling within an acceptable range are examined for sampling error. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual

techniques will be documented and used in future training sessions or individual re-training.

Note: If two people are involved in collecting a duplicate, each person should filter his or her own sample and not filter the other person's sample.

WVDEP Laboratory Quality Assurance Program

All water quality samples must be analyzed by a laboratory that has been approved thru the WVDEP Laboratory Quality Assurance Program. The Quality Assurance Program is responsible for certifying environmental laboratories to ensure that the DEP receives accurate and reliable analytical data. Laboratories are certified when they follow approved methods, employ well-trained capable staff, and use equipment and instrumentation suited to the work they perform.

Certified laboratories are grouped into **three** categories.

1. **Commercial** – stand-alone laboratories testing samples for a fee.
2. **Municipal** - laboratories associated with publicly owned treatment works operated by cities or public service districts.
3. **Industrial** - manufacturing company owned and operated laboratories.

The majority of water quality samples collected by the Watershed Branch will be analyzed by certified commercial laboratories. However, sometimes it may be necessary to use a certified municipal laboratory due to holding times combined with distance to a commercial lab (*e.g.,* fecal coliform samples). In addition, industrial laboratories may be used when cooperating with industry partners in certain studies.

The WVDEP maintains an up-to-date list of approved laboratories on the web located at: http://www.dep.wv.gov/WWE/Programs/lab/Pages/default.aspx